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Plasma Chemistry Reference Values in Free-living Bonelli's Eagle (*Hieraaetus fasciatus*) Nestlings

Javier Balbontín[1] and Miguel Ferrer
Department of Applied Biology, Estación Biológica de Doñana, Consejo Superior de Investigaciones Científicas, Avda de Maria Luisa s/n, Pabellón del Perú, Sevilla 41013 Spain

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Few studies dealing with plasma biochemistry and physiology in wild birds can be found in the scientific literature. Most research papers deal with domestic or captive birds kept in zoos, rehabilitation facilities, or research centers (Lewandoski et al. 1986, Redig 1991, Ferrer 1993, Dobado-Berrios et al. 1998). The knowledge of normal reference values in plasma for wild species is very important to a wide range of multidisciplinary subjects. Veterinarians need to have this information in order to better diagnose the condition of wild birds entering into rehabilitation centers or zoos (Lepoutre et al. 1983, Cooper et al. 1986). Also, information gathered from hematological research is of a great importance for ecologists because such data may provide insights into the health of individuals being studied. Body condition that is related to other ecological factors such as survival, fecundity, or habitat quality, could be estimated by levels of urea, uric acid, and other blood parameters (Cherel et al. 1987, Ferrer et al. 1987, García-Rodriguez et al. 1987, Robin et al. 1987).

It is important to know normal blood parameter reference values for endangered species involved in a reintroduction or restoration program in order to better understand the physiological status of the released birds. Normal reference values in blood chemical constituents are known only for 5% of bird species which have been studied mostly in captive situations (Ferrer 1993).

Although most of the information available comes from captive birds, it might be expected that a captive condition might affect hematological values (Bell and Freeman, 1971, Miglirioni et al. 1973, Wolf et al. 1985, Sturkie 1986, Ferrer et al. 1987, García-Rodriguez et al. 1987). Factors such as age or sex influence the total variation found in plasma enzymes, proteins, metabolites, and other organic molecules. However, presently these factors are poorly understood due to difficulty of gathering information on different age-classes in wild species. Other factors affecting values of chemical components in plasma are circadian rhythm (García-Rodriguez et al. 1987), seasonal changes (Wolf et al. 1985), or plasma storing methods (Bustamante and Traviani 1993).

The Bonelli's Eagle (*Hieraaetus fasciatus*) is an endangered species that has suffered a rapid population decline in most areas of Europe including Spain (Cugnasse 1984, Palma et al. 1984, Hallmann 1985, Arroyo et al. 1990). In this article, we present normal chemical plasma values found in a free-living endangered population of nestlings of this bird of prey. Data from 21 biochemical substances (including metabolites, total protein, inorganic ions, and enzyme activities) and differences found between sexes in this age-class are reported. In addition, we have examined the differences found between free-living and captive birds of this long-lived raptor.

Methods

We have studied a breeding population of Bonelli's Eagles in the province of Cádiz that is located in southern Spain (5°32′W, 36°41′N). We collected blood from both nestlings of a free-living population of south Spain and from captive young Bonelli's Eagles. The diet of eagles in our region included a preponderance of rabbits (*Oryctolagus cuniculus*), and Red-legged Partridge (*Alectoris rufa*) (Gil-Sánchez et al. 1994, Ontiveros and Pleguezuelos 2000). Birds kept in captivity were fed ad libitum with partridge and rabbit. Blood collected from free-living nestlings was taken when individuals were between 47–53-d-old, about 10 d before fledging. One of us climbed or descended to several nests each year to band and measure young; at the same time 2 ml of blood was extracted from the brachial vein of the wing. To minimize circadian variations of blood parameters, we extracted all blood samples between 1100–1500 H. CST blood was collected in lithium-heparin tubes and the plasma was separated by centrifugation (10 min: 907.2 × g). Cellular fraction and plasma samples were immediately frozen (−80°C) Analyses were carried out 4 mo later with a Hitachi 747 multichannel automatic analyzer (Tokyo, Japan) with the reagents recommended by Boehringer-Mannheim (Darmstadt, F.R.G). Plasma was analyzed (abbreviations and methods indicated in parentheses) for amylase (AMY; maltoheptaose reaction), cholesterol (CHOL; cholesterol esterase), creatinine (CREA; Kinetic Jaffé reaction), creatinine kinase (CK; optimal standard method DGKC), glucose (GLUC; hexokinase method), aspartate aminotransferase (AST; DGKC technique), alanine aminotransferase (ALT; DGKC technique), total protein (TP. biuret reaction), triglycerides (TRIG; enzymatic method), urea (UREA; urease method, uric acid (UA, uricase method), alkaline phosphatase (AP; paranitrophenylphosphate method), colinesterase (CHE), L-lactate dehydrogenase (LDH; SFBC technique), bilirubin (BILIR; DPD method), calcium (Ca; cresolphtalein complexone reaction), phosphorus (iP; molybdenum blue reaction),

[1] E-mail address: Balbonja@ebd.csic.es

sodium (Na, Indirect potentiation, E. Selective), potassium (K; Indirect potentiation, E. Selective), magnesium (Mg; blue xilidil reaction).

The cellular fraction of the blood sample was used to sex all eagles. For this analysis, primers 2945F, cfR and 3224R were used following Ellegren (1996). The total number of eagles sampled included 28 nestlings, 14 females and 14 males, belonging to a free-living population and two young males and three young females that were captive birds.

We used the Student's *t*-test to check for differences in the means of blood parameters between sexes and between captive and free-living birds. Non-parametric tests were employed for those variables not meeting either normality or homoscedasticity assumptions (Siegel and Castellan 1988). When data were not normally distributed, they were $\log_e$ transformed (Sokal and Rohlf 1995). After this transformation only two blood parameters AP and K, did not exhibit a normal distribution. The assumption of homoscedasticity was met for those parameters showing a normal-like distribution. Levene's F test was used to test for homoscedasticity. All tests were two-tailed and statistical significance was set at $P < 0.05$. Means are given with ±SD. Range for all variables are also provided. In some cases due to the small plasma volume of some samples, it was not possible to do analyses of all chemical parameters, thus, the sample size is not the same for all blood components. Analyses were performed with the Statistica Ax 99 package (Statistica. 1996. Version 5. Statsoft, Inc.).

RESULTS

Normal reference plasma chemistry values for nestling Bonelli's Eagles are shown in Table 1. We found differences in parameters between male and female free-living nestlings in two of 21 plasma parameters measured. Males showed higher glucose levels in blood and lower AST activity than females (Table 1). We found that urea ($t = 3.78$, df = 32, $P < 0.001$), uric acid ($t = 3.21$, df = 33, $P < 0.001$), alkaline phosphatase ($t = 3.52$, df = 32, $P < 0.001$) and creatinine kinase ($t = 2.5$, df = 32, $P < 0\ 05$) values were lower than these measured in captive eagles. However, glucose ($t = -3.89$, df = 33, $P < 0.001$) was higher in captive compared with free-living birds.

DISCUSSION

Our data showed that there were differences in plasma glucose levels between sexes in nestling Bonelli's Eagles, males showing higher levels than females (Table 1). Polo (1995) failed to find any difference in this parameter between sexes in eight avian orders including Falconiformes. However, Polo (1995) only examined birds that were in captivity. Levels of glucose in plasma have been correlated with metabolic rate (Umminger 1977). Birds that showed a high metabolic rate, because of high activity such as flying with fast flapping, would also have higher levels of glucose in plasma. Bonelli's Eagle exhibits a strong sexual size dimorphism, with females being much larger than males, which implies that male and female nestlings of this species would have different metabolic rates due to different growth patterns. Consequently, each sex may be exposed to a different energy demand during the nestling period. For example, female nestlings gain an estimated 7.3 grams more than males each day during the nestling period, which lasts on average between 59 (Minguez et al. 2001) and 63 d (Real et al 1998). Therefore, males might have higher glucose levels because they have a lower growth energy demand than females. The larger females might channel more glucose into tissue formation than smaller males. Recently, Casado et al. (2002) also found that male nestling Booted Eagles (*Hieraaetus pennatus*) had higher glucose than females, another raptor with strong sexual size dimorphism. However, González and Hiraldo (1991) studying free-living nestling Marsh Harriers (*Circus aeruginosus*) (also shows sexual size dimorphism), found the reverse tendency with female nestlings having higher glucose levels than males. A possible explanation is that glucose level in nestlings could indicate the quantity and quality of food received by each individual during the growing period. Parents might be able to allocate food in an asymmetric way within brood favoring either sex depending on environmental condition or food availability; for example, the larger or older sibling may receive more food in years of scarcity (Mock et al. 1987).

As most of the knowledge in normal plasma reference values comes from captive birds, we compared blood chemistry values from our free-living nestling sample with values gathered from five juvenile captive eagles. These birds were young eagles in their first year; therefore, they were a few months older (less than a year different) than eagles sampled in nature. For this reason, age may have affected the differences found between these two groups Among the differences we found were those related to nitrogen residues. Captive birds had lower UREA and UA than free-living ones. These chemical constituents have been associated with physical condition in birds of prey. An increase in plasma levels of these nitrogen residues has been predicted when birds are subjected to a food stress situation (Ferrer 1994, Alonso-Alvarez and Ferrer 2001). Therefore, lower levels in UREA and UA in captive birds indicate that these eagles were in better body condition than free-living birds.

In this study we also found that captive eagles showed higher plasma glucose levels than free-living ones, which might be in accordance to what has been reported in other birds (Lewandoski et al. 1986, Casado et al. 2002). Captive individuals also showed lower CK activity than free-living ones. This enzyme mediates in muscle contraction and is related with physical activity. Thus, it seems reasonable that eagles in captivity would show lower CK activity than free-living eagles. The difference found on AP activity between captive and free-living birds might be attributed to an age effect rather than to a captive condition since it is well known that the activity of this enzyme decreased with aged in birds of prey. Concretely, this enzyme is related to the ossification of frontal

Table 1. Blood chemistry values for male and female nestling Bonelli's Eagles from Cádiz, Spain.

Variable	(SI Unit)	Males			Females					
		Mean ± SD	Range	*N*	Mean ± SD	Range	*N*	*t*	df	*P*[a]
GLU	(mmol L^{-1})	15.74 ± 2.09	(12.06–18.50)	14	14.03 ± 1.39	(11.3–15.7)	14	2.53	26	0.017*
UREA	(mmol L^{-1})	2.27 ± 0.56	(1.33–3.17)	13	2.52 ± 0.98	(0.92–4.33)	14	−0.84	25	0.40
UA	(μmol L^{-1})	684.2 ± 279.6	(434.3–1362.5)	14	827.0 ± 307.6	(321.3–1362.5)	14	−1.51	26	0.14
CREAT	(μmol L^{-1})	24.8 ± 4.43	(17.7–35.4)	14	25.6 ± 3.54	(21.2–32.7)	14	−0.44	26	0.66
PT	(g/l)	29.8 ± 1.5	(26–32)	10	30.1 ± 2.8	(27–36)	10	−0.36	18	0.71
AP	(Ul L^{-1})	2148 ± 696	(509–3160)	13	2280 ± 327	(1696–2759)	14	−0.63 (1)	25	0.52[b]
CHE	(UI L^{-1})	1191 ± 272	(961–1860)	9	1140 ± 228	(789–1571)	10	0.45	18	0.65
AMY	(UI L^{-1})	1148 ± 303	(784–1776)	9	942 ± 194	(726–1310)	12	1.90	19	0.07
CK	(UI L^{-1})	3859 ± 883	(2116–5034)	13	3853 ± 918	(1880–5352)	14	0.01	25	0.98
LDH	(UI L^{-1})	1647 ± 160	(1489–1903)	6	1828 ± 355	(1199–2417)	8	−1.15	12	0.27
COL	(mmol L^{-1})	4.35 ± 0.60	(3.06–5.54)	16	4.53 ± 0.73	(3.29–5.80)	14	−0.65	26	0.51
TG	(mg/dl)	71.3 ± 35.1	(28–166)	13	81.7 ± 36.6	(36–161)	14	−0.93	25	0.35
MG	(mmol L^{-1})	0.77 ± 0.04	(0.54–0.70)	6	0.72 ± 0.07	(0.54–0.79)	8	−1.67	12	0.12
NA	(mmol L^{-1})	66.09 ± 1.27	(65–67.7)	5	65.95 ± 1.81	(62.2–68.1)	8	0.13	11	0.89
K	(mmol L^{-1})	22.3 ± 11.9	(0.89–28.1)	5	21.2 ± 11.7	(1.15–28.6)	8	0.21 (1)	11	0.82[b]
CA	(mmol L^{-1})	2.15 ± 0.25	(1.63–2.38)	6	2.20 ± 0.23	(1.80–2.45)	8	−0.25	12	0.80
P	(mmol L^{-1})	1.55 ± 0.23	(1.2–1.94)	7	1.49 ± 0.19	(1.10–1.68)	8	0.54	13	0.59
BILIR	(μmol L^{-1})	1.19 ± 0.17	(0.85–1.70)	6	1.02 ± 0.51	(0.51–2.22)	8	0.44	12	0.66
AST	(UI L^{-1})	171.6 ± 18.2	(153–202)	6	203.2 ± 31.5	(139–241)	8	2.17	12	0.04*
ALT	(UI L^{-1})	11.5 ± 2.2	(10–16)	6	13.3 ± 3.8	(9–19)	8	−1.04	12	0.31
GGT	(UI L^{-1})	14.5 ± 14.5	(7–53)	9	15.1 ± 10.0	(5–39)	12	−0.41	19	0.67

[a] Probability that means are equal calculated with a Student's *t*-test.

[b] Probability that distributions are equal calculated with a Mann-Whitney *U*-test.

* Significantly different, $P < 0.05$.

bones that take place throughout the immature to adult age period (Dobado-Berrios and Ferrer 1997, Viñuela et al. 1991).

RESUMEN.—Este articulo presenta información sobre los valores de referencia normales de parámetros bioquimicos presente en plasma sanguineo de pollos estudiados en libertad de Aguila perdicera (*Hieraaetus fasciatus*). Investigamos diferencias en los parámetros sanguineos entre sexos. Los machos muestran unos niveles más altos de glucosa y una actividad enzimatica de Alanino-amino transferasa (AST) mayor que las hembras. Los pollos fueron comparados con jóvenes águilas mantenidas en cautividad. Las aguilas cautivas tuvieron valores más bajos de urea, ácido úrico, fosfatasa alcalina, creatinina quinasa y valores más altos de glucosa que los pollos marcados en libertad.

[Traducción de los autores]

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